Prevalence of circulating *T. pallidum* DNA and RNA in PK™TP+/ FTA•ABS + blood donors

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Scientific Question

- Do blood donors with confirmed positive syphilis tests have evidence of circulating *Treponema pallidum*? If so, what is the prevalence?

Theoretically, all confirmed positive syphilis tests should represent current or past disease.

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Hypothesis

- Confirmed positive syphilis tests do not represent current infection.

How did we arrive at this hypothesis?

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Background

- Anecdotal evidence from blood donors who have been notified of confirmed positive syphilis tests.
- Evidence in the literature that in low risk populations, most (if not all) positive results represent antibody from previous disease or biological false positive reactivity.

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continued

- Conditions associated with biological false positive test results can affect all of the tests currently in use for screening of donated blood (PK™TP, FTA-ABS and RPR).

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Assumption

- An individual with spirochetemia is not likely to present as a blood donor.
  - ✓Syphilis is a rare disease in the US (in 1998, CDC reported an incidence of 2.6/100,000 population (0.5/100,000 in Whites)
  - ✓Peak spirochetemia occurs during the secondary phase, which presents as acute, symptomatic disease.

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Assumption (continued)

✓ There has not been a documented case of transfusion transmitted syphilis in over 30 years, despite the fact that:

1. spirochetemia may occur during the primary phase

   - this phase may be asymptomatic and seronegative early (last reported case was from a seronegative donor) and

2. transfusion transmitted syphilis would result in secondary phase syphilis that should be recognizable

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Goal

- Determine if there is any evidence of circulating *T. pallidum* in the blood of donors who are PK™TP reactive, FTA-ABS positive by specific detection of DNA or RNA (as surrogate measures of potential infectivity).

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Sample

- Target sample size: 100 PK™TP reactive, FTA-ABS positive donations; 50 RPR reactive, 50 RPR non-reactive
- Use existing platelet concentrates from these donations

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Platelet Concentrates

- *T. pallidum* spirochetes are likely to segregate with white blood cells (WBC's)
- Preparation of platelet concentrates yields both concentrated platelets, and concentrated white blood cells
  - ✓ whole blood=10^9 WBC's/500 ml=2 x 10^6/ml
  - ✓ packed red blood cells=10^8 WBC's/250 ml=4 x 10^5/ml
  - ✓ platelet concentrates=10^7 WBC's/50 ml=2 x 10^5/ml

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Testing

- PCR for *T. pallidum* specific DNA using the pol A gene target
  - ✓capillary electrophoresis and fluorescent detection
  - ✓read on an ABI 310 Genetic Analyzer
  - ✓sensitive to 10-25 organisms/100 ul platelet concentrate extracted

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Testing (continued)

- Multiplex PCR kit (Roche) for *T. pallidum, H. ducreyi* and HSV 1&2 DNA
  - ✓47kD basic membrane protein gene target for *T. pallidum* previously described.
  - ✓Sensitive to 10 organisms/100 ul platelet concentrate extracted

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Testing (continued)

- RT-PCR using 16S rRNA template for reverse transcription production of cDNA
  - ✓detection by Southern blot or Agilent Bioanalyzer
  - ✓sensitive to 1 organism/140 ul platelet concentrate extracted

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Testing (continued)

- Controls
  - ✓DNA: both assays included internal and external control samples. Positive external controls were diluted to 50 organisms per 100 µL from stock *T. pallidum* (Nichols strain) cultures.
  - ✓ RNA: positive controls diluted to 10^{-1} genome equivalents per 140 µL from stock *T. pallidum* (Nichols strain) cultures.
  - ✓Negative controls: all assays

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Results

- 100 samples tested negative for *T. pallidum* DNA by both assays.
- 100 samples tested negative for *T. pallidum* RNA

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Study limitations

- The optimal sample for detection is fresh whole blood.
- Because we can never "prove" a negative test result, in a pilot study with a sample size of 100 and all negative test results, there is up to a 3% chance that there is an incorrect interpretation of no evidence of infectivity.

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Discussion

- There are differences in findings between this study and the CDC work presented today.
- There are differences in the populations studied (blood donors vs individuals identified during a syphilis outbreak).
- Results of a case control study: ~50% of blood donors with a confirmed positive test result report a previous history of syphilis (> 1 yr prior to donation)

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Conclusions

- We did not demonstrate circulating *T. pallidum* DNA or RNA in the platelet concentrates of PK™TP reactive, FTA-ABS positive blood donors in this pilot study.
- It is unlikely that the blood of donors with confirmed positive syphilis test results is infectious for syphilis.

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Relationship of anti-HBc and Serologic Tests for Syphilis (STS) to Blood Donor Behavioral Risk Factors

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Retrovirus Epidemiology Donor Study (REDS)

Background - anti-HBc

- Poor specificity and high donor loss (0.7 - 1.8%) when used for screening of donated blood
- Value for detection of HBV infection is limited
- Surrogate value for behavioral risk detection is speculated, but unknown

Background - STS

- Screening tests for syphilis (STS) have been performed on blood donations since 1938
- No well-documented cases of transfusion-transmitted syphilis in the US in over 30 years
- Surrogate value for behavioral risk detection is speculated, but unknown

Background - STS (cont.)

- 1995 NIH Consensus Conference debated the value of continued blood donor STS screening
- August 1999: FDA seeks data regarding the value of donor STS (Proposed Rules: Requirements for testing....)
  - as a marker of high risk behavior
  - as a surrogate test for other infectious diseases
  - in preventing the transmission of syphilis through blood transfusion

Objective

- Assess the value of anti-HBc and STS as surrogate indicators of blood donor risk behaviors

REDS 1998 Donor Survey

- ARC, Greater Chesapeake and Potomac Region
- ARC, Southeastern Michigan Region
- ARC, Southern California Region
- Blood Centers of the Pacific - Irwin/UCSF
- Oklahoma Blood Institute
- New York Blood Center
- Blood Bank of San Bernardino
- Lifeblood (Memphis)

- Medical Coordinating Center - Westat, Inc.

REDS 1998 Donor Survey (cont.)

- Anonymous mail survey
- Allogeneic donors; ≥18 years.
- Monthly probability sample of donors April through October 1998.
- 92,581 sampled donors at eight sites
- 57% survey response rate

REDS 1998 Donor Survey (cont.)

- Survey sample included four laboratory test strata:
  - anti-HBc+
  - STS+
  - other lab reactivity
  - seronegative
- all anti-HBc+ and STS + donors surveyed

REDS 1998 Donor Survey - Content

- Demographics
- Donation history/experiences
- Deferrable Risk Assessment (DR)
- Multiple Investigations
  - » Surrogate value of STS and anti-HBc
  - » Incentives
  - » Hemochromatosis
  - » HIV test-seeking

DEFERRABLE RISK

- A risk that should have resulted in deferral according to blood donor screening criteria at the time of the survey

Results: Deferrable Risk (DR)

	DR Prev	OR	Adj.OR*
◆ Neg	2.9%	1.0	1.0
◆ anti-HBc	8.0%	2.9 †	2.7†
◆ STS+	13.7%	5.4 †	5.5†
◆ Other+	11.5%	4.4 †	3.3

* Odds ratios adjusted for gender, age, race/ethnicity, education, center, FT donors (all p< .001)

† $p < 0.001$

Proportion of Overall DR Associated with anti-HBc and STS (%)

	DR Prev	% of Overall DR
◆ Neg	2.9	94.4
◆ anti-HBc	8.0	2.4
◆ STS+	13.7	1.0
◆ Other+	11.5	2.2

Proportion of Overall MSM and IDU risks associated with anti-HBc and STS (%)

	MSM	s/MSM	IDU	s/IDU
◆ Neg	94.1	96.5	87.0	93.7
◆ anti-HBc	3.0	2.1	2.5	1.9
◆ STS+	0.3	0.5	0.2	0.5
◆ Other+	2.6	1.0	10.3	3.9

Proportion of Overall STS-related risks associated with anti-HBc and STS (%)

	STS+/12 mos.	Rx for S/G
◆ Neg	62.4	89.4
◆ anti-HBc	5.7	3.8
◆ STS+	31.9	5.6
◆ Other+	0.0	1.3

Results: Deferrable Risk (DR) excluding STS

	DR Prev	OR	Adj.OR*
◆ Neg	2.7%	1.0	1.0
◆ anti-HBc	7.3%	2.9 †	2.7†
◆ STS+	4.7%	1.7 †	5.5†
◆ Other+	11.5%	4.6 †	3.3

* Odds ratios adjusted for gender, age, race/ethnicity, education, center, FT donors (all p< .001)

† p < 0.001

Summary anti-HBc+

- When controlled for FT donor status and demographic factors, anti-HBc+ donors have a 2.6-fold higher level of reported deferrable risk than seronegative donors. When anti-HBc prevalence is considered, anti-HBc+ is associated with 2.4% of overall DR)
- Qualitatively, anti-HBc-associated risks are similar to those of the overall donor base.

Summary STS+

- When controlled for FT donor status and demographic factors, STS+ donors have a 5.2-fold higher level of reported deferrable risk than seronegative donors. When STS+ prevalence is considered, STS is associated with 1.0% of overall DR)
- **However, DR associated with STS+ is mostly due to STS-related risk factors.**

Conclusions

- Results of this study indicate that the value of STS as a surrogate behavioral risk measure is inconsequential.
- **If parallel molecular studies continue to show an absence of *T pallidum* in STS+ blood, the requirement for STS testing of donated blood should be removed.**

Study Limitations

- Survey risk estimates are reproducible, but are based upon self-report. Accuracy has not been validated by other independent measures.